IJLST

ISSN: 0974-5335
IJLST (2011), 4(1):1-6

In Vitro Vegetative Propagation of Blepharis Molluginifolia, Pers. – A Medicinal Plant

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ABSTRACT:
An efficient reproducible in vitro vegetative propagation protocol has been developed for *Blepharis molluginifolia*, Pers. (Acanthaceae) by using nodal explants. Direct shoot formation from nodal segments was obtained on MS medium with BAP (0.5 mg/L) within 4 weeks. The in vitro shoots thus obtained were successfully rooted in MS media supplemented with IAA (0.5 mg/L) alone. Further, the well developed *in vitro* grown plantlets were transferred into plastic cups containing soil, vermiculites and sand (1:2:1) and incubated in aseptic culture room for 10 days. All the in vitro grown plants survived and they were successfully acclimatized in soil.

Key words: Direct organogenesis, nodal explants, BAP, IAA.

INTRODUCTION

Tissue culture techniques offer several possibilities for plant propagation by using vegetative plant parts to induce organogenesis and plant regeneration under aseptic condition. Therefore, vegetative propagation is one of the potential and useful methods that need to be tried for plant species, which are medicinally important and difficult to raise through seeds and other means. Plant propagation through vegetative means multiplies plants and preserves their essential genetic characters. This is an easy and effective technique for multiplication and conservation of plant species. Sexual reproduction is considered less important than vegetative propagation for arctic and alpine species [1]. Plant growth regulators and other chemicals are widely used in vegetative propagation to improve rooting and subsequent growth of cuttings [2],[3].

Blepharis molluginifolia Pers. is a threatened medicinal and prostrate herb which belongs to the family Acanthaceae. It is commonly known as Haridachchu in Kannada. The leaf of this plant is mainly used for skin diseases, urinary discharges, bone setting and allergies.[4]. Therefore, the aim of the study presented here is to develop a protocol for *in vitro* vegetative propagation, which will be of great value as an alternate method and to conserve this plant species *ex situ* through *in vitro* propagation to obtain large number of plants within a short period of time.

MATERIALS AND METHODS

Plant materials and explants sterilization:

Blepharis molluginifolia plants were collected from the Karnatak University campus Dharwad. Collected plant materials grown and maintained in the well established greenhouse of Department of Botany, Karnatak University, Dharwad, India for source of explants. Excised nodal segments (0.5-1.0 cm) of young healthy plants were used for *in vitro* culture. The nodal explants were first thoroughly washed under running tap water for 15-20 min and then treated with liquid detergent (Tween-20) for 5-10 min. Later the explants were washed with double distilled water for 5 min. After explants immersed in 70% (v/v) ethanol for 2-3 min. and washed with sterile glass double distilled water for 2-3 times. Eventually, the explants were treated with aqueous solution of 0.1% (w/v) $HgCl_2$ for 1-2 min and rinsed with sterile glass double distilled water for 2-3 times in order to remove traces of mercuric chloride residues. The sterilized explants were aseptically inoculated on to MS Medium.

Culture media and growth condition:

Murashige and Skoog medium supplemented with 3% (w/v) sucrose and the pH of the medium was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl then solidified with 0.8% agar prior to autoclave at 121^0C at 15 lbs pressure for 15-20 min. Inoculations were done under aseptic conditions in a laminar air flow cabinet, where cultures tubes (150 X 25 mm) containing 20-25 ml medium and plugged tightly with non-absorbent cotton. All cultures were incubated in a controlled-environmental growth chamber at 25±2 ^{0}C under 16 hrs photoperiod at 3,000 lux light intensity (40W white fluorescent tubes, Philips, India) and with 55- 60 % relative humidity.

RESULTS AND DISCUSSION

A protocol for *in vitro* vegetative propagation of *Blepharis molluginifolia* has been developed through by using various explants viz. node, internode, leaf, root were used as an explants, but the maximum response were obtained through nodal explant cultures on MS medium supplemented with BAP 0.5 mg/L.

Survey of literature suggests that BAP is the most reliable and useful Cytokinins for shoots in higher plants. Many workers were successful in their attempts for shoot induction by using BAP (1mg/L) in *Aloe polyphylla* [5] and BAP (1mg/L) in *Aloe vera*. [6]. When nodal explants were cultured on MS Medium containing different concentrations and combinations of Cytokinins (BAP & Kn), direct shoot was induced from nodal explants (Table 1. Fig 1. A & B). The shoot regeneration efficiency in short period and it was confirmed by using BAP (0.5 mg/L) alone showed better response. The same hormone concentration has

been reported for *Hypericum mysorense* (BAP in 1.0 mg/L) [7].

Then, maximum shoot elongation up to 8.85 cm were obtained in the same MS medium within 4 weeks of duration (Fig. 1B) in BAP (0.5 mg/L) alone, but when BAP in combination with auxins like IAA in same concentration showed shoot elongation up to 4.82 cm only but response is about 70 % of cultures. Hence, BAP in different concentrations more effective in induction of axillary shoots and Kn alone were considerably less effective in number of shoots and average length of shoots/explant was much lower (Table 1). This indicates at the hormonal variations comprising of BAP alone and in combination with IAA had a positive effect on both number and length of shoots induced by culturing the nodal segments of *B. molluginifolia*. (Table.1 and Fig. 1C).

The percentage of explants showing induction of number of shoots per culture increased gradually with an increase of concentrations of BAP from 0.2-0.5 mg/L. Further, an increase in its concentrations ranging from 0.5-1.5 mg/L, did not show any effect and but numbers of shoots are reduced per culture. Therefore, standardization of an *in vitro* culture system for particular species is necessary and most significant findings in the present investigation is MS medium with BA (0.5 mg/L) alone was essential for the *in vitro* plant regeneration.

In the present investigation, the highest level of BAP (1.5 mg/L) the explants failed to produce more shoots but 0.5 mg/L BAP is more suitable than 0.5 mg/L Kn for shoot formation. Similar development of rapid regeneration from nodal explants has been reported for *Ocimum bascilicum* (BAP in 0.2 mg/L) [8], *Adhatoda Vasica* (BAP in 0.5 mg/L) [9]. *Ocimum gratissimum* (BAP in 0.5 mg/L) [10], *Sida cordifolia* (BAP in 2.0 mg/L) [11], *Lens culinaris* (BAP in 2 mg/L) [12], *Gynura procumbens* (BAP in 2 mg/L) [13].

Induction of direct organogenesis has also been reported in a number of medicinal plants, it variably depends on the appropriate level of both endogenous and exogenous hormones as in *Digitalis lanata* [10], *Pinellia ternate* [14], *Rehmannia glutinosa* [15], [16]. It is in the present study on *B.molluginifolia*, the nodal explants showed direct organogenesis when they cultured on MS medium supplemented with BAP alone in 0.5 mg/L.

Rooting of regenerated shoots:

The maximum *in vitro* root induction was induced from shootlets obtained from nodal explants on MS media supplemented with 0.2-1.5 mg/L of IAA & NAA. Among IAA and NAA at different concentrations, IAA was found to be most effective in 0.5 mg/L tested for root induction on *in vitro* raised shoots (Table 2). Among the different concentrations of IAA tested, 0.5 mg/L IAA was indicated as optimum for proper rooting in which 100 % shoots were rooted within 8 weeks in the same MS media of culture (Fig 1. C & D).These findings are also recorded for other plants species *Hypericum mysorense* (IAA in 0.5 mg/L) [7]. *Phyla nodiflora* (NAA in 1.5 μM) and *Leptadenia reticulata* (IAA in 3 μ*M*) [17], *Stevia rebaudiana* (IAA in 0.1 mg/L) [18] and *Vanasushava pedata* (IAA in 2.0 mg/l) [19]. In the present study the maximum rooting (75%) of culture augmented with IAA in 0.5 mg/L. About 5.3 cm long thick roots were obtained from shoots on MS media with same hormone (IAA 0.5 mg/L). Though the rooting observed in MS culture media with (NAA 0.1-1.5 mg/L) but the response was 15 % only.

CONCLUSION

Presently described method can be successfully employed for the large scale production of plants through *in vitro* vegetative propagation of *Blepharis molluginifolia*, which is a rare perennial prostrate herb and is in threatened status in South India. The use of MS media with low concentration of Cytokinins and Auxins has shown maximum response for induction of shoot and root from nodal explants.

The acclimatization of *in vitro* grown plants was accomplished for a short duration.

Thus, this is the best possible non conventional low cost efficient *in vitro* vegetative propagation method which helps in restoration of this important medicinal plant within a short period of time and can be made available throughout the year for the use in the field of medicine.

ACKNOWLEDGEMENT

Authors acknowledge the Chairman, P.G. Department of Botany, for extending facilities to conduct this work in the department and also UGC New Delhi for financial support as RFSMS, for one of the authors.

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ISSN: 0974-5335
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Table 1. Effect of various concentrations and combinations of growth regulators on MS medium for direct shoot induction from nodal explants of *Blepharis molluginifolia*, Pers.

MS with Growth regulators (mg/l)			% of culture induced shoot	Average No. of shoots/culture (cm)	Average length of shoots (cm)
BAP	**Kn**	**IAA**			
0.0	0.0	0.0	00	Nil	Nil
0.1	0.0	0.0	00	Nil	Nil
0.2	0.0	0.0	30	1.5	5.43
0.5	**0.0**	**0.0**	**75**	**2.0**	**8.85**
1.0	0.0	0.0	60	1.41	7.77
1.5	0.0	0.0	45	1.11	6.20
0.5	0.0	0.1	55	1.18	3.88
0.5	0.0	0.2	65	1.30	4.5
0.5	0.0	0.5	70	1.42	4.82
0.5	0.0	1.0	55	1.0	4.15
0.5	0.0	1.5	45	1.1	3.64
0.0	0.1	0.0	0	Nil	Nil
0.0	0.2	0.0	15	1.0	0.63
0.0	0.5	0.0	20	1.2	0.7
0.0	1.0	0.0	10	1.16	0.5
0.0	1.5	0.0	5	1.0	0.3

Data was recorded after 6 weeks after incubation.

Table 2. Effect of various concentrations of Auxins on MS media for induction of root from *in vitro* regenerated shoots of *Blepharis molluginifolia*.

MS + Auxins (mg/l)	% of culture induced roots	Average No. of roots/culture	Average length of roots (cm)
IAA			
0.1	00	Nil	Nil
0.2	30	4.3	2.8
0.5	**75**	**5.3**	**4.2**
1.0	60	2.6	1.5
1.5	45	2.4	1.2
NAA			
0.1	0	Nil	Nil
0.2	5	1	0.2
0.5	15	1.3	0.35
1.0	10	1	0.3
1.5	5	1	0.2

Data was recorded after 8 weeks after incubation.

IJLST

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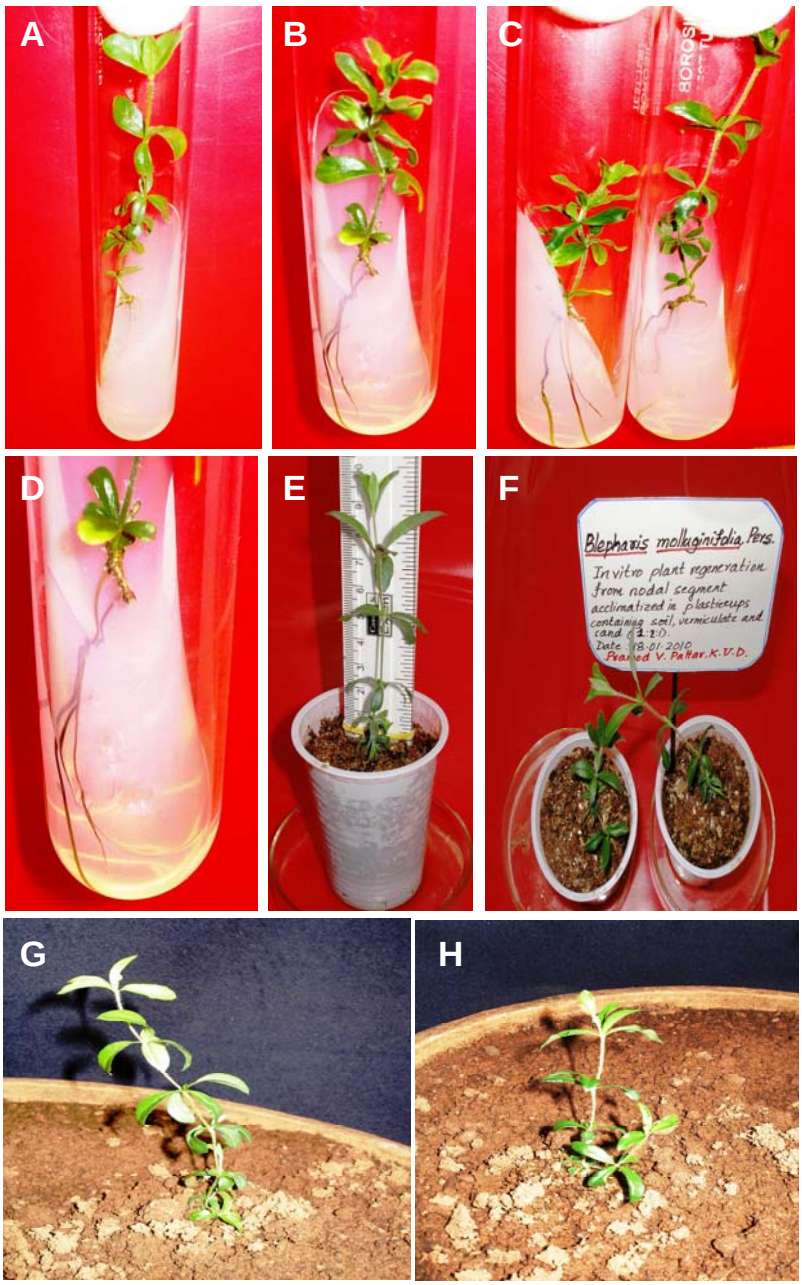

Fig.1.A -Regeneration of shoot from nodal explants after six weeks of incubation.

B, C - Shoot and Root differentiation after 6 weeks of incubation.

D - Induction of root from shoot after 8 weeks of incubation.

E -Elongated hardened plant in plastic cups containing vermiculites.

F - *In vitro* grown plants in plastic cups with soil, vermiculites and sand (1:2:1).

G, H- *In vitro* plantlets in earthen pots.

Fig.1. Showing *in vitro* vegetative propagation of *Blepharis molluginifolia*, Pers.

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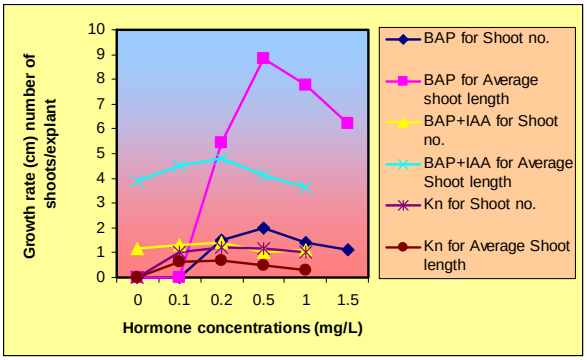

Fig.2. *In vitro* response of Nodal explants on MS medium with different concentrations and combinations of Cytokinins and Auxins.

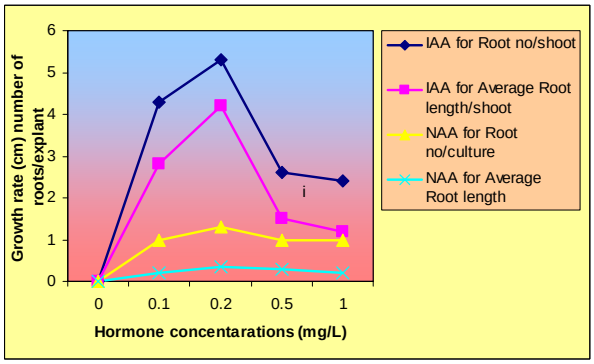

Fig. 3. *In vitro* regenerated shoot induced root on MS medium with the different concentrations of Auxins.

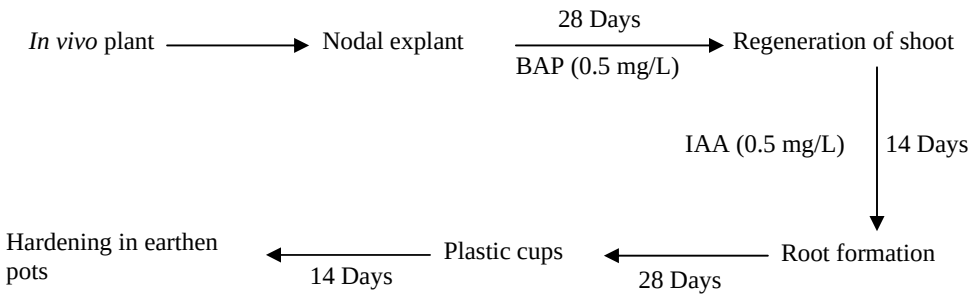

Fig. 4. A schematic representation of *in vitro* vegetative propagation of *Blepharis molluginifolia*, Pers.

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